J. Water Environ. Nanotechnol., 6(3): 277-293 Summer 2021

ORIGINAL RESEARCH PAPER

Detection of Carcinogenic By-Products in Effluents Produced by Biodigester Septic Using of Array Sensor and Nanostructured Polymers: Evaluation and Characterization

Eduarda Regina Carvalho*, Wilson Tadeu Lopes da Silva

Brazilian Agricultural Research Corporation -Embrapa Instrumentation Center, CP 741, 13.560-970, São Carlos-SP, Brazil

Received: 2021-04-17 Accepted: 2021-06-10 Published: 2021-07-01

ABSTRACT

A sensory system was manufactured and tested to detect carcinogenic organochlorines, such as trihalomethanes (THM), in effluents produced by a biodigester septic tank. Gold-coated interdigitated microelectrodes were coated with various nanostructured conducting polymers, single or mixed, under different conditions and configurations. Polymer films were assembled by the self-assembly technique and their impedance was analyzed in a frequency range from 1 Hz to 1 MHz. The results of the sensory system, analyzed by the PCA Main Component Analysis, allowed us to identify the discrimination of the different samples tested. Good discrimination between sensors was also observed, when PCA's were obtained, in contamination experiments with standard solutions of total trihalomethanes (TTHM), obtaining a total variance (PC1 = 98.02%; PC2 = 1.98%) of the observations. The sensory system based on global selectivity using an interdigitated electrode and nanostructured conducting polymers allowed the statistical discrimination of samples. The future expectation is to update the system and implement monitoring of nanostructured sensors based on the reuse of domestic effluents, mainly for agricultural applications or for other purposes, in a sustainable way.

Keywords: *sewerage, water reuse, sensorial system, nanostructured polymer, trihalomethanes*

How to cite this article

Regina Carvalho E., Tadeu Lopes da Silva W. Detection of Carcinogenic By-Products in Effluents Produced by Biodigester Septic Using of Array Sensor and Nanostructured Polymers: Evaluation and Characterization. J. Water Environ. Nanotechnol., 2021; 6(3): 277-293.

DOI: 10.22090/jwent.2021.03.008

INTRODUCTION

The environmental control of water is a major concern of governments and research centers that study appropriate technologies for the monitoring and efficient use of resources, as well as exploring the possibilities of reusing water, in order to ensure that irreparable damage does not occur, improving the quality of life for current and future generations. However, when evaluating the deficient sanitary infrastructure existing in the world, especially in developing countries, the monitoring and reuse of sewage water, for example, lacks studies and the development of treatment and monitoring techniques aimed at its safe reuse.

Latin America is the region with the freshest water on the planet: Brazil, Colombia, and Peru are among the ten countries with the greatest amount of water resources. Despite this, 490 million Latin Americans still do not have a bathroom at home and 34 million do not have constant access to clean water, according to a study by the World Bank and

* Corresponding Author Email: *carveduarda@gmail.com*

100 million people without access to adequate sanitation systems, according to UN data [1-3].

Mainly in underdeveloped countries, sewage is discharged into bodies of water, in the open air and into the oceans and, in fact, the discharge of sanitary sewage without prior treatment, into a given body of water, can cause deterioration of water quality, causing ecological, social and economic impact, generating potential risks to the health of the population, contributing even more to the water crisis that devastates the planet. Adequate sanitation separates human waste from the environment, preserving the system and ensuring a better quality of life for the local population [4].

The application of sewage to the soil, for example, constitutes one of the oldest forms of final disposal of sanitary sewage [4;5]. It appeared as a form of treatment but aroused the interest of farmers for its application in agriculture. For years, this practice has become inadvisable due to the presence of pathogens and public health concerns. However, the problems of water scarcity and the increase in research on techniques for the safe and controlled application of wastewater - in agriculture - resurfaced interest in the subject [6], being treated and used with criteria.

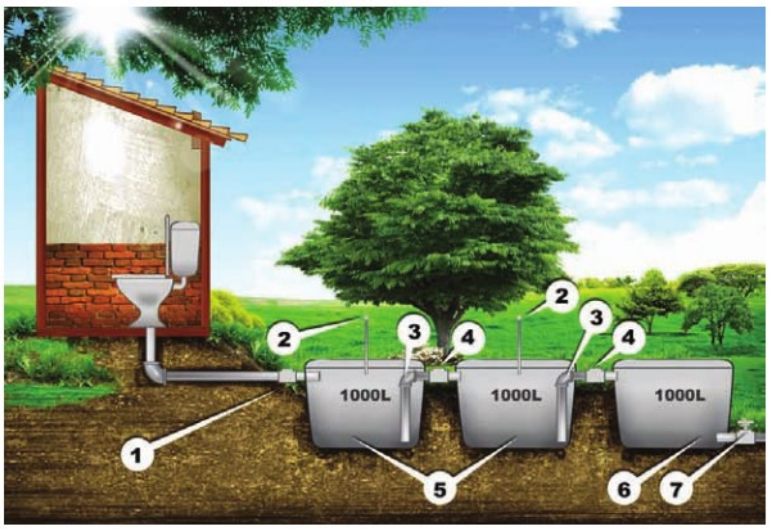
An important point that favors the use of treated sewage effluents in the irrigation of crops is the fact that the soil, together with the plants, acts as a "living filter", absorbing and retaining nutrients and pollutants [7]. Furthermore, the application of conventionally treated domestic sewage supplies the soil mainly with nitrogen and phosphorus, thus reducing the total amount of commercial fertilizers used, which can mean greater profits for farmers [8; 9].

The use of sewage effluents in irrigation, despite being an attractive and popular alternative, is not a risk-free practice and has constituted a challenge to Soil Science [7;10 and 11]; either because of the complexity of understanding the behavior of the constituents of the effluent in the soil or because of the formation of toxic by-products after treatment, as well as the dangers of pollution. Factors that affect the quality of effluent for use include the quality at the generating source, the reliability of the treatment process, as well as the design and operation of distribution systems. Sanitary, technical and economic aspects are fundamental when seeking sustainability for the use of effluents. Disinfection of sanitary sewage using chlorine, for example, in gaseous or hypochlorite form, is a simple solution, with a relatively low cost. However, it has been the subject of reservations, based on the finding that this agent generates potentially carcinogenic by-products [12-15].

Historically, chlorine has been the most used oxidant for disinfecting water sources. Research carried out in recent years has shown, however, that this technology is inefficient in eliminating some epidemic microorganisms, such as giardia, Cryptosporidium, and viruses in municipal effluents [16-17]. In contact with dissolved organic matter, chlorine promotes the formation of toxic by-products, harmful to man and aquatic biotas, such as organochlorine compounds [18-20] called trihalomethanes (THM), where the most expressive are: chloroform, bromoform, bromodichloromethane, and dibromochloromethane. Other chlorination by-products are also identified, such as haloacetic acids, haloaldehydes, halo ketones, halophenols, TOX (total organic halides), among others, capable of producing potentially adverse and dangerous effects on human health, with carcinogenic potential [21].

Although the current guide value for the total of organochlorines, such as trihalomethanes present in water, is 80 mL^{-1} according to the WHO (which is based on the extrapolation of the appearance of tumors in rats, when subjected to drinking water ingestion with chloroform within two years); in Brazil, only from 1990 onwards, by Ordinance No. 36, of January 19, 1990, of the Ministry of Health, was it established a maximum concentration of 100 paras mL^{-1} for THM [22]. The seriousness of the problem is such that chloroform (one of the main THMs) generates chronic effects associated with carcinogenicity, in concentrations of the order of 2 mgL^{-1}, with positive results in bioassays for the development of cancer. The formation of tumors by chloroform is strongly associated with cytotoxicity and proliferation of regenerative cells in tissues, which has been considered a determining factor for its carcinogenicity [22-24].

It is known that the chlorination of sanitary sewage is more complex than that of supply water, requiring dosages above 10 mgL^{-1} to reach a concentration of coliforms below 100/100 mL. The degree of toxicity of chlorination is related to the concentration and composition of the total residual chlorine (free or combined), presence of organic compounds, pH, temperature, and contact time [21]. Another problem with the application



1 – Retention valve
2 – Relief Chimney
3 – Curve of 90°
4 – "T" of inspection
5 e 6 – 1000mL box
7 – Register

Fig. 1. Structure of the biodigestive septic tank system.*Font: Adapted from Novaes, 2002.*

of chlorine in wastewater disinfection is the need for dechlorination. If the effluent is released without dechlorination in rivers or lakes, chlorine can negatively affect the biological balance of the receiving body [25]. There are several other types of disinfectants, which are efficient but costlier and that generate other types of by-products [26].

However, in sewage disinfection, chlorination presents itself as a very attractive alternative, due to its low operating cost, as well as the full domain of the application of this technology in our environment, especially due to the experiences arising from the drinking water supply systems and even so, being carried out with reservations regarding its use due to the formation of toxic by-products [27-29].

Acher et. al. [30], state that the disinfection of treated domestic effluents is mandatory before its use in crop irrigation, aquifer recharge through infiltration, or before its disposal in the soil or large bodies of water. Rebhun et.al. [27] mention that the chlorination of treated effluents is practiced in several parts of the world, being particularly important in compartments that include the recovery or reuse schemes, such as in irrigation.

In Brazil, half of the rural population does not have access to adequate sanitation. This means that more than 20 million people are at greater risk of contracting infectious or parasitic diseases, which are acquired mainly through contact with untreated sewage and/or contaminated groundwater, through the use of rudimentary cesspits ("black pit", well or hole). In this sense, a system, developed by the Brazilian Agricultural Research Corporation (Embrapa), at the Instrumentation Center, is presented as a way to enable the treatment of domestic sewage in rural areas, being a simple and low-cost system for the population rural [31].

The treatment system, called as Biodigester Septic Tank (BioSepT), promotes the anaerobic treatment of feces and urine through two fermentation chambers, made up of 1000 L water tanks connected by a siphon system and an effluent collection box. The inoculant is the newly collected cattle or sheep manure, whose microflora is capable of digesting any type of food contained in these wastes, transforming the effluent obtained free of pathogenic germs (salmonellas, shigellas, and others) and also unpleasant odors [32;18], (Fig. 1). After the disinfection process, the generated effluent can be disposed of in the soil [31-32].

The inadequate or uncontrolled application of residues to the soil can significantly affect the soil itself, groundwater, and surface and even bring public health problems. Studies have shown that in Sewage Treatment Plants, the concentration may be small due to the selectivity of the reaction with ammonia and the lower reaction rate with THM-forming compounds, in the presence of chlorine (free or combined) [33].

Effluents containing ammonia when chlorinated at doses of up to 18 mgL^{-1} form about 300 mL^{-1} of dissolved organohalogenates (OHD). In nitrified effluents. when chlorinated at the same dose, they form up to 526 mL^{-1} of THMs and 2.840 mL^{-1} of (OHD) [34-35]. Rossi et al. (2005) [34] showed that in the disinfection of raw, anaerobic, facultative, and post-treated sewage with sodium hypochlorite there was no formation greater than 35 mL^{-1} of THMs, probably due to the preferential reaction of chlorine with ammonia nitrogen or due to the chemical characteristics of the precursors existing in the tested effluents. However, due to the by-products generated by the combination of chlorine with other substances present in the effluents, by-products such as trihalomethanes can also be formed, which bioaccumulate in the food chains [36].

Developing technologies to monitor and quantify potentially carcinogenic disinfection by-products (whether in sewage effluents or raw and distributed water) is as important as treating what is already polluted, developing "clean" processes with minimal waste generation, thus avoiding the production of more effluents to be treated. Furthermore, they are actions that must precede any decision that implies any change in treatment systems. Furthermore, state-of-the-art technologies must necessarily be accessible, so that actions such as these are carried out, especially for the countless municipalities and rural properties in underdeveloped countries.

The detection of chemical species through sensors is today one of the most investigated areas in science and technology, due to its great importance and variety of applications. The study of conducting polymers has aroused enormous scientific and technological interest due to the need for active materials for sensors in numerous applications. These polymers become electroactive through a doping process with a variety of substances, which allows an increase in their versatility for use in sensor devices [37].

The main qualities required in a sensor, in general, are high sensitivity, stability, and selectivity. Quality control and assessment of the number of pollutants found in the air, soil, and water requires sensors that can detect particular substances in a specific and selective way. However, the sensors available on the market, manufactured with inorganic semiconductors such as tin oxide, despite being cheap, are not very selective. Thus, new alternatives have been investigated in the field of sensors, and in this context, organic molecules have been explored [38], among which conductive polymers stand out [37].

The use of electrochemical sensors ("electronic tongues") based on the concept of global selectivity to analyze the composition of the beverage [39]. Sensors based on this concept have been used in environmental analyses [40] and water quality control [41-44], being an efficient tool of monitoring.

Thus, the objective of this work was to test electrochemical sensors made of nanostructured conductive polymers, in treated sewage effluents, and contamination tests, based on electrochemical impedance, to determine their sensitivity for the detection of carcinogenic compounds, such as THM(s), in the search for the development of an effective and efficient device. Samples of effluents generated in a BioSepT installed on a farm were used. This work describes a new proposal for the evaluation and differentiation of treated sewage effluents, through a sensory system based on global selectivity, observed by an array of interdigitated microelectrodes coated with conducting polymers of nanostructured ultra-thin films, presenting as a differential the development of a viable and innovative system for the detection of carcinogenic organochlorine by-products in these systems, as well as its possible applicability in the monitoring and detection of environmental contaminants resulting from aquatic chlorination.

MATERIAL AND METHODS

Ultra-thin films of conductive polymer nanostructure

Following the methodology described by Carvalho et al (2016) [45], the polymers used for film fabrication were polyaniline (PANI), poly(o-ethoxyaniline) (POEA), aquatic humic substances (AHS), and sulfonated lignin (SL). POEA were chemically synthesized as described in references [46-48], using ammonium peroxydisulfate in HCl 1.0 $molL^{-1}$ at 0°C. The PANI was synthesized analogously to the synthesis method of MacDiarmid, A.G. et al, [49]. SL was obtained from Melbar (Brazil) and AHS was isolated from a water sample collected from River João Pereira, which is a tributary of the River Itapanhaú, close to the city of Bertioga, on the south coast of São Paulo state, Brazil [50]. The extraction and fractioning were made according to procedures established by the International Humic Substances Society [51],

Table 1. Sensing units in the sensor array to analyze effluents.

Sensing Unit	Type of material	Symbology
S1	----------	Without Film
S2	One layer of poly(o-ethoxyaniline)	POEA
S3	A bilayer of POEA and sulfonated lignin (SL)	POEA/SL
S4	One layer was deposited from a complexed mixture of POEA and SL (in the same solution)	POEA + SL
S5	One layer of SL deposited for 3 min	SL
S6	One bilayer of aquatic humic substances (AHS) and POEA, with each layer being deposited for 3 min	AHS/POEA
S7	One layer obtained from a complexed mixture of POEA and AHS	POEA+ AHS
S8	One layer deposited from a polyaniline (PANI) solution at pH = 5.0	PANI
S9	One layer deposited from a AHS solution	AHS
S10	One bilayer of PANI and SL	PANI/SL

as well as recommendations by Malcolm [52]. The AHS solutions were prepared in the concentrations of 5, 10, and 30 mg L^{-1}. All the aqueous solutions of POEA, SL, and AHS were prepared using ultrapure water from a Milli-Q system (Millipore®). The pH was adjusted by adding amounts of HCl 0.1 mol L^{-1} or NH_4OH 0.1 mol L^{-1}. The details for film deposition for the sensing units are presented in Table 1.

The polymer nanostructured films with different architectures were deposited onto the interdigitated microelectrodes of glass-coated gold using the layer-by-layer (LbL) technique [53-54]. The layer-by-layer self-assembly technique (LbL) is one of the most common and inexpensive techniques employed to produce thin films, with good control of thickness and molecular architecture, where different mechanisms can be used to build up multilayer films. Consists of the adsorption of molecular layers via electrostatic interaction, which can be also, driven by hydrogen bonds or bio-specific interactions [55].

The layers were deposited with an immersion time of 3 min. per layer, from aqueous solutions at pH = 5.0 and concentration 10^{-3} mol L^{-1}, except PANI. As it is insoluble in aqueous media, PANI was dissolved in NMP (N-Methyl-2-Pyrrolidone) at a concentration of 10^{-3} mol L^{-1}, for the manufacture of the polymeric films. Ten sensing units were produced as follows: i) Sensor 1 (S1): without film; ii) Sensor 2 (S2) had one layer of POEA deposited from a pH = 5 solution onto the electrode; iii) Sensor 3 (S3): a bilayer of POEA and SL (POEA/SL), with each layer being deposited for 3 min.; iv) Sensor 4 (S4): one layer was deposited from a complexed mixture of POEA and SL (in the same solution) (POEA+SL); v) Sensor 5 (S5): one layer of SL deposited for 3 min. (SL); vi) Sensor 6 (S6): one bilayer of AHS and POEA, with each layer being deposited for 3 min. (AHS/POEA); vii) Sensor 7 (S7): one layer obtained from a complexed mixture of POEA and AHS (POEA+AHS); viii) Sensor 8 (S8): one layer deposited from a PANI solution at pH = 5.0 (PANI); ix) Sensor 9 (S9): one layer deposited from an AHS solution (AHS); x) Sensor 10 (S10): one bilayer of PANI and SL, with each layer being deposited for 3 min. (PANI/SL), second, Carvalho et al., (2016), [45]. Studies on deposition layers with experimental observations by micrographs using POEA / PANI / SL are described in other publications authored by Carvalho et al., [56].

The treatment of the electrochemical data had been made by principal component analysis (PCA). PCA is a mathematical transform that is used to explain variance in experimental data. This mathematical tool allows discriminating different samples of origin in bi-dimensional graphs, locating each sample in a specific quadrant. This discrimination allows creating a database where the electrochemical characteristics will be associated with the physicochemical results [57-59]. The data were interpreted with an equivalent circuit, from which the capacitance of the film was chosen as the variable to be treated with principal component analysis (PCA), using StatSoft Statistica version 10.0. A computer program for data acquisition was developed by Embrapa – Instrumentação de São Carlos/SP.

Fig. 2 (a) and 2(b), illustrates in a simplified way, a microelectrode and the sensory system assembled with the different deposited nanostructured polymers. The analysis was carried through by electrochemical impedance using the Solartron impedance analyzer SI 1260, Fig. 2(c), being this

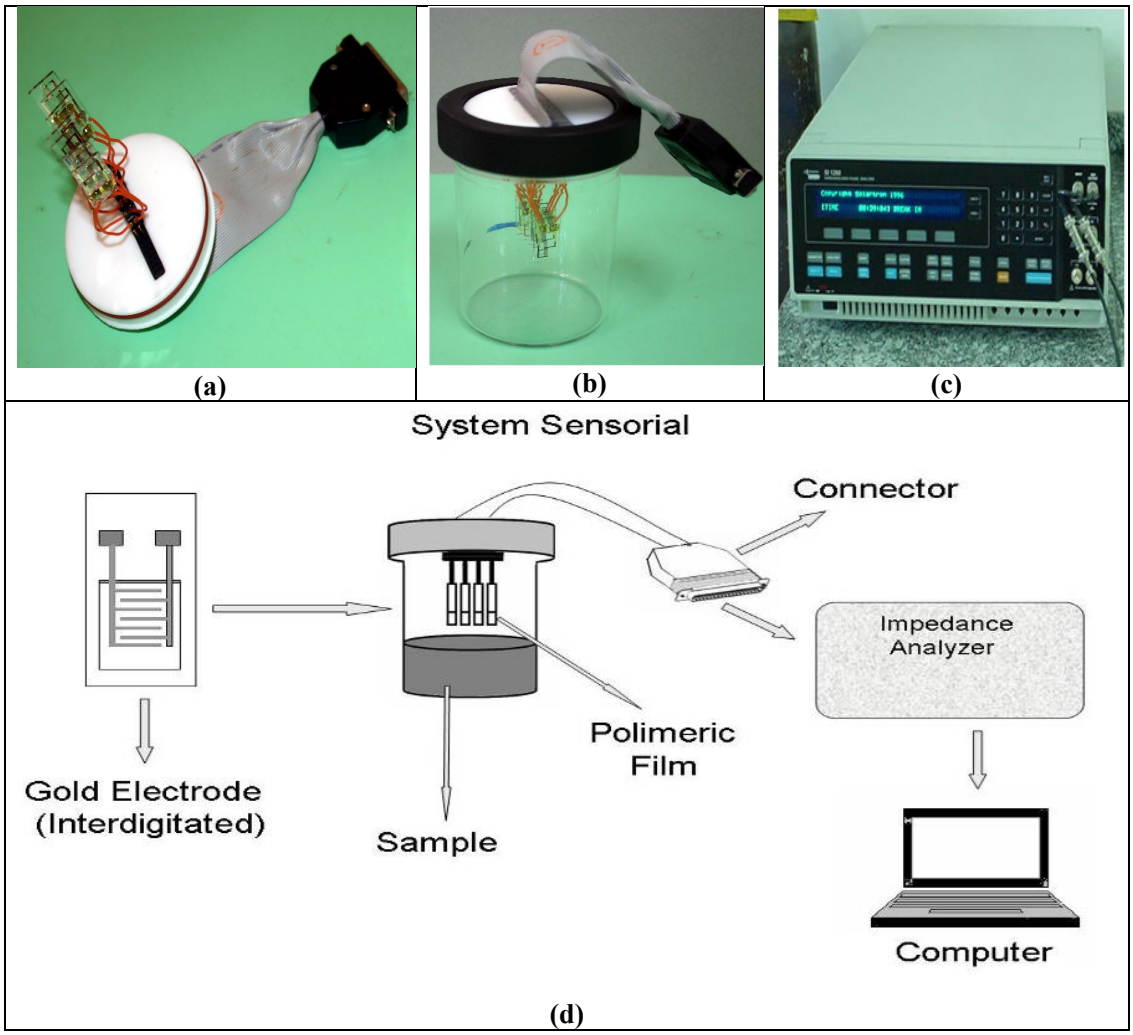


Fig. 2. (a) Microelectrode; (b) Sensory system assembled with the different deposited nanostructured polymers and he sample holder; (c) Solartron impedance analyzer SI 1260 and (d) Data acquisition scheme.Font.: Authors.

interface to a microcomputer for the data acquisition. The frequency and the amplitude of the signal had been fixed in 1 kHz and 50 mV, respectively, after a study of the performance of each sensor, according to Carvalho et al., (2016), [45]. Fig. 2(d) illustrates the data acquisition scheme.

Location of Collect Area

The samples used were collected at São João ranch, located in the rural area of the municipality of São Carlos, state of São Paulo, on the border between São Carlos and the municipality of Itirapina (Fig. 3). The city of São Carlos is located in the interior of the state of São Paulo - Brazil coordinates 22° S 47° 53' 27" W, has a hot climate with dry winter, within a tropical altitude zone. Average daily temperatures ranged from 5,5 to 28.1°C, monthly from 17.3 to 23.6°C and annual averages from 20.8 to 21.7°C.

The São João ranch is located on the banks of the Ribeirão Feijão watershed (Fig. 4), downstream from one of the water catchment points in the municipality of São Carlos. There, an Environmental Education Program is developed, with the development of monitored visits with children, with activities related to the themes of effluent treatment, recycling, composting, as well as visits to the Permanent Preservation Area (APP) of the property, with activities related to planting seedlings to rebuild the local forest. Part of the watershed of this river is located within the Guarani Aquifer, considered the largest freshwater

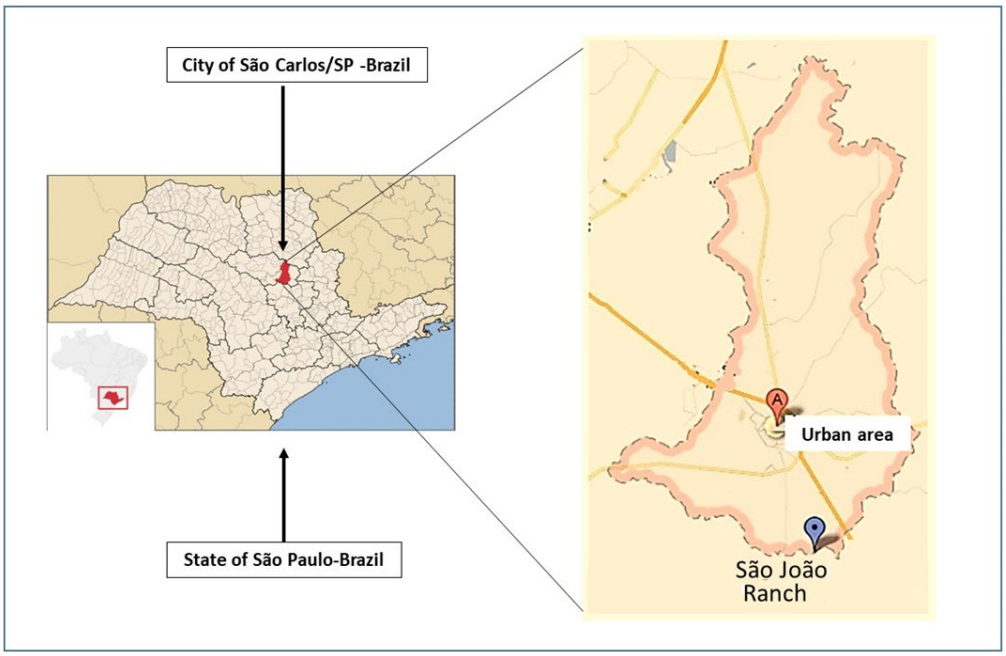


Fig. 3. Location of Ranch São João in the city of São Carlos-São Paulo, Brazil, adapted of Google Maps.

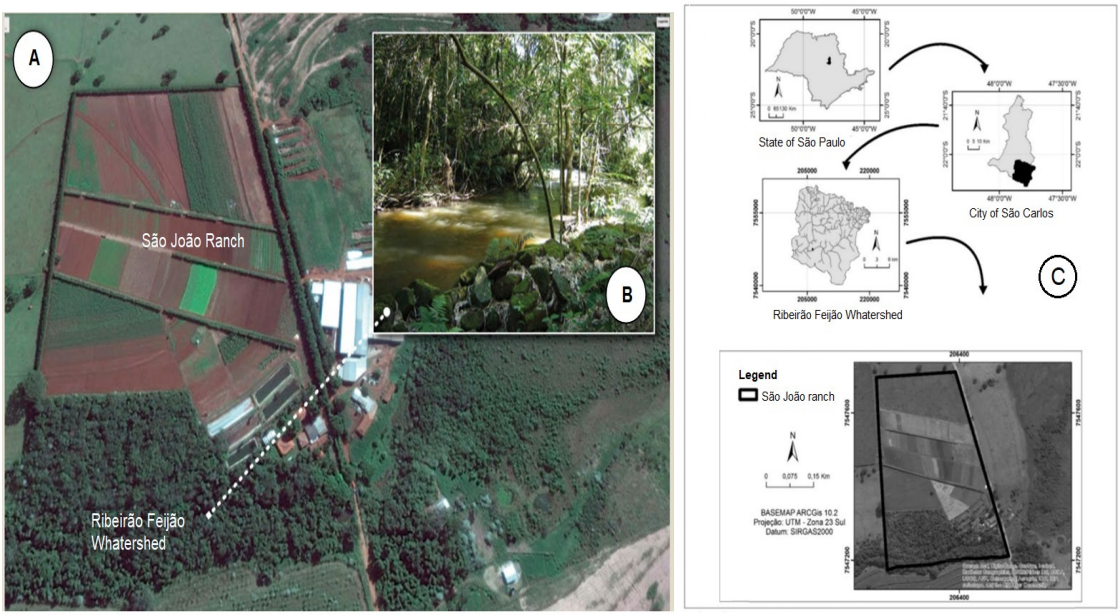


Fig. 4. São João Ranch: (A) Panoramic view of the ranch, (B) Ribeirão Feijão watershed and (C) Location of São João ranch. In this figures (A) and (B) were adapted from Almeida-Neto, et al., (2020) [61] and (C) adapted from Silva et al., (2016), [62].

aquifer in the Paraná Basin. This stream provides approximately 35% of the drinking water for the Municipality of São Carlos, being an important source for the city. In Fig. 5 below, the points where effluent sample collections were performed are highlighted.

The BioSepT

The BioSepT, present on the ranch, is connected to all the toilets on the site, treating an average volume of 1000 liters every five days. This biodigester cesspool consists of four water tanks of 1000 liters each, and the application of cattle

Fig. 5. São João Ranch: (1) Residence on the ranch; (2) Biodigestor septic tank system; (3) Ribeirão Feijão watershed and (4) Artesia post of the Guarani Aquifer. Adapted from Iniciativa Verde (2015), [63].

Fig. 6. In (I) Filter Garden; (II) biodigestor septic tank installed and in (III) Ribeirão Feijão watershed margem, adapted from Hernandes (2021), [64].

manure is carried out only in the first box and the last two were installed for the storage of treated effluent (Fig. 6). Next to the water tanks, there is a Filtering Garden, which is an alternative to properly dispose of sewage from sinks, tanks, and showers, rich in soaps, detergents, food waste, and grease (the so-called "grey water"). The scarcity of water resources demonstrates the importance of

Table 2. Nomenclature assigned to collected samples.

Nomenclature	Definition
A1	Water house
A2	water sample from the garden entrance filter
A3	water sample from the filtering garden outlet
A4	effluent sample from the BioSepT outlet
A5	Sample collected from the Guarani Aquifer artesian post

reusing this water, which has several applications: irrigation of crops, washing floors and windows, use in the toilet, among others.

Physical-Chemical Parameters

Sample pH, dissolved oxygen, conductivity, and temperature values were determined using a WTW Multiparameter probe model Multi340i. Turbidity was determined using a portable microprocessor turbidity meter model HI 93703 (Hanna Instruments) and residual chlorine was determined using a portable model HI-95711 meter (Hanna Instruments). The readings were taken 3 times, and the arithmetic mean of the results was calculated, with their respective deviations.

Sterile bags were used to collect water samples, with identification tape and sterile closing wire. The samples taken to the laboratory were placed in 100ml plastic bags. Plastic bags containing sodium thiosulfate tablets were used to collect the chlorinated water. Sodium thiosulfate tablets are used to neutralize halogenated compounds (such as chlorine), allowing effective bacterial and organic testing. For the analysis of the residual chlorine content, Exstik Cl200A Chlor Meter chlorine meters, from Extench Instruments and model HI-95711 (Hanna Instruments) were used. The effluents produced by the BioSepT and other samples were named according to Table-2 below.

Contamination experiments

Com o intuito de se verificar a resposta do sistema sensorial, quanto a presença dos trihalometanos, em solução, foram realizados experimentos de contaminação utilizando solução padrão de trihalometanos (THM): clorofórmio, bromofórmio, bromodiclorometano e dibromoclorometano, of the Supelco. Concentrações de 20mL de cada THM, foram adiconadas em solução de 100 mL de água ultrapura, resultando solução total de 80mL do total de trihalometanos (TTHM).

RESULTS AND DISCUSSION

Physical-Chemical Parameters of Samples

Table 3 below shows the physicochemical parameters obtained for the samples. In that table, for sample A1 referring to tap water in the house, there was an average of 7.41±0.75 mgL^{-1} related to the oxygen content. The conductivity was in the order of 1344.33±25.79 $mScm^{-1}$ at a temperature of 28.40±0.62 °C. The sample presented a basic pH of around 7.00, turbidity of around 0.29±0.07 FTV with a redox potential of around 21.66±1.52 mV. The concentration of residual chlorine detected was in the order of 0.12±0.01 mgL^{-1}. For sample A2 referring to the effluent at the entrance to the garden, it was observed an average of 8.18±0.07 mgL^{-1} related to the oxygen content. The conductivity was in the order of 1616.66±22.89 $mScm^{-1}$ at a temperature of 26.66±0.15°C. The sample had an acid pH of around 4.81, turbidity of around 92±1.52 FTV with a redox potential of around 112.66±4.16 mV. The concentration of residual chlorine detected was in the order of 0.39±0.02 mgL^{-1}.

For sample A3 referring to the effluent at the exit of the garden, it was observed an average of 5.06±0.29 mgL^{-1} referring to the oxygen content. The conductivity was in the order of 1602.00±22.60 $mScm^{-1}$ at a temperature of 25.06±0.05°C. The sample had an acid pH of the order of 5.22±0.02, turbidity of the order of 38.94±0.66 FTV with a redox potential of the order of 91.66±2.88 mV. The concentration of residual chlorine detected was in the order of 0.28 mgL^{-1}. For sample A4, referring to the effluent at the exit of the pit, an average of 5.59±0.14 mgL^{-1} was observed, referring to the oxygen content. The conductivity was in the order of 3.45±0.07 $mScm^{-1}$ at a temperature of 25.63±0.40°C. The sample presented a basic pH in the order of 8.05, turbidity in the order of 35.53±1.39 FTV with a redox potential in the order of -80.33±0.57mV. The concentration of residual

Table 3 Physicochemical parameters obtained.

Sample	Parameter	Measure 1	Measure 2	Measure 3	Arithmetic average
A1	Residual Chlorine (mgL^{-1})	0.10	0.15	0.12	0.12±0.01
	Oxygen (mgL^{-1})	6.54	7.79	7.90	7.41±0.75
	Conductivity (μScm^{-1})	1323	1337	1373	1344.33±25.79
	Temperature (^0C)	29.1	28.2	27.90	28.4±0.62
	pH	7.13	6.95	6.83	6.97±0.15
	Turbidity (FTV)	0.35	0.31	0.21	0.29±0.07
	Redox Potential (mV)	22	23	20	21.66±1.52
A2	Residual Chlorine (mgL^{-1})	0.37	0.40	0.42	0.39±0.02
	Oxygen (mgL^{-1})	8.27	8.15	8.12	8.18±0.07
	Conductivity (μScm^{-1})	1591	1624	1635	1616.66±22.89
	Temperature (^0C)	26.80	26.70	26.50	26.66±0.15
	pH	4.83	4.81	4.81	4.816±0.01
	Turbidity (FTV)	92.00	91.00	94.00	92.33±1.52
	Redox Potential (mV)	116.00	108.00	114.00	112.66±4.16
A3	Residual Chlorine (mgL^{-1})	0.26	0.28	0.31	0.28±0.02
	Oxygen (mgL^{-1})	5.35	5.07	4.77	5.06±0.29
	Conductivity (μScm^{-1})	1577.00	1608.00	1621.00	1602±22.60
	Temperature (°C)	25.00	25.10	25.10	25.06±0.05
	pH	5.19	5.23	5.24	5.22±0.02
	Turbidity (FTV)	38.21	39.12	39.50	38.94±0.66
	Redox Potential (mV)	95.00	90.00	90.00	91.66±2.88
A4	Residual Chlorine (mgL^{-1})	0.41	0.44	0.43	0.42±0.03
	Oxygen (mgL^{-1})	5.74	5.58	5.45	5.59±0.14
	Conductivity (μScm^{-1})	3.37	3.47	3.51	3.45±0.07
	Temperature (°C)	26.10	25.40	25.40	25.63±0.40
	pH	8.05	8.05	8.06	8.05±0.00
	Turbidity (FTV)	36.12	36.54	33.95	35.53±1.39
	Redox Potential (mV)	-80.00	-80.00	-81.00	-80.33±0.57
A5	Residual Chlorine (mgL^{-1})	*nd	*nd	*nd	*nd
	Oxygen (mgL^{-1})	8.75	8.34	7.52	8.20±0.62
	Conductivity (μScm^{-1})	1850	1873	1883	1868.66±16.92
	Temperature (°C)	29.20	28.60	28.40	28.73±0.41
	pH	7.18	7.04	6.76	6.99±0.21
	Turbidity (FTV)	0.00	0.18	0.00	0.60±1.03
	Redox Potential (mV)	38.00	40.00	43.00	40.33±2.51

**nd= not detected*

chlorine detected was in the order of 0.42±0.03 mgL^{-1}.

For sample A5, referring to water taken from the artesian well (source of the entire system), an average of 8.20±0.62 mgL^{-1} regarding the oxygen content. The conductivity was in the order of 1868.66±16.92 $mScm^{-1}$ at a temperature of 28.70±0.41 °C. The sample had a basic pH of around 7.00, turbidity of around 0.60±1.03 FTV with a redox potential of around 40.33±2.51 mV. In this sample, it was not possible to detect the concentration of residual chlorine, possibly because it was below the detection limit of the measuring equipment.

Sensory response to different samples

The different depositions and conditions of the polymers, for the different sensors in response to the effluent samples from the BioSepT, can be seen in Figs. 7a,7b,7c, and 7d. Plotting the score factors on the same PC1-PC2 plane (Fig. 7) shows a clear separation between the sensors.

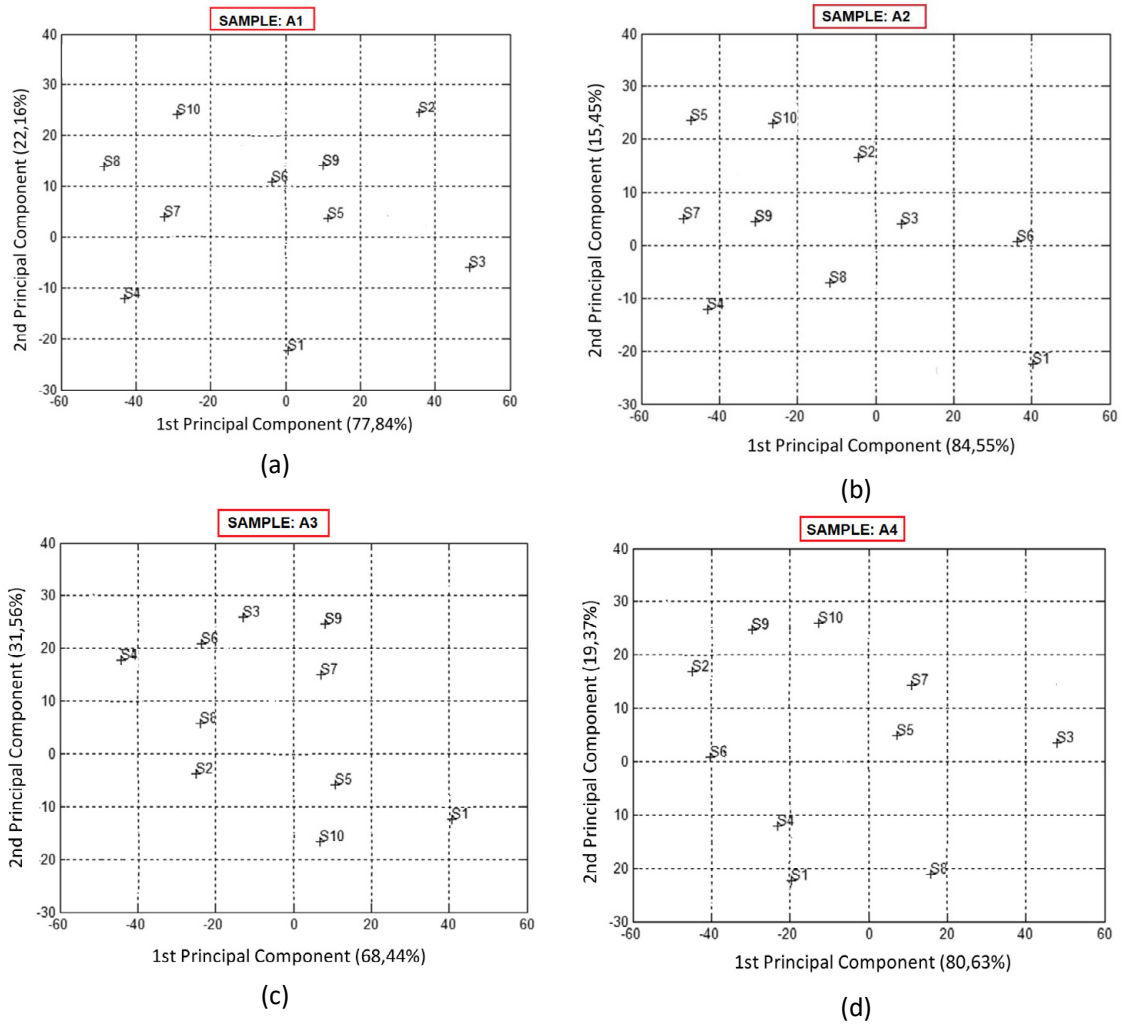


Fig. 7. Analysis of the Main Component (PCA) obtained for the sensors in the presence of effluent samples, referring to the different polymeric complexes deposited on the interdigitated microelectrodes. In (a) sample A1, (b) sample A2, (c) sample A3 and (d) sample A4.

The sensory system was able to differentiate the samples in different compositions, interacting in different ways with the polymeric films, producing different capacitance values in each sensor. The values for the first and second components represent the following values of the total variance of the observations: (a) Sample A1: PC1 = 77.84 %; PC2 = 22.16%, (b) Sample A2: PC1 = 84.55 %; PC2 = 15.45%, (c) Sample A3: PC1 = 68.44 %; PC2 = 31.56%, (d) Sample A4: PC1 = 80.63 %; PC2 = 19.37% and (e) Sample A5: PC1 = 75.27 %; PC2 = 24.73% (data not shown).

Fig. 8 shows the sensory analysis of samples A1, A2, A3 and A4 compared to the standard solution containing the TTHM (total THM). The plotting of the scoring factors on the same plane PC1-PC2 (Fig. 8) shows a clear separation between the samples, the sensory system being able to differentiate them. The selectivity of the sensors about the samples is observed, with excellent distinction in the first main component. The sensors responded in a particular way for each sample, according to the interaction of the sensory arrangement. The values of the first and second components represent approximately 100% of the total variance of the observations. In, Fig. 8, it is observed that PC1 = 88.02% and PC2 = 11.98%. These results suggest that sensors respond selectively when in contact with solutions.

Also in Fig. 8, it is possible to observe the points referring to each sample, which tend to group

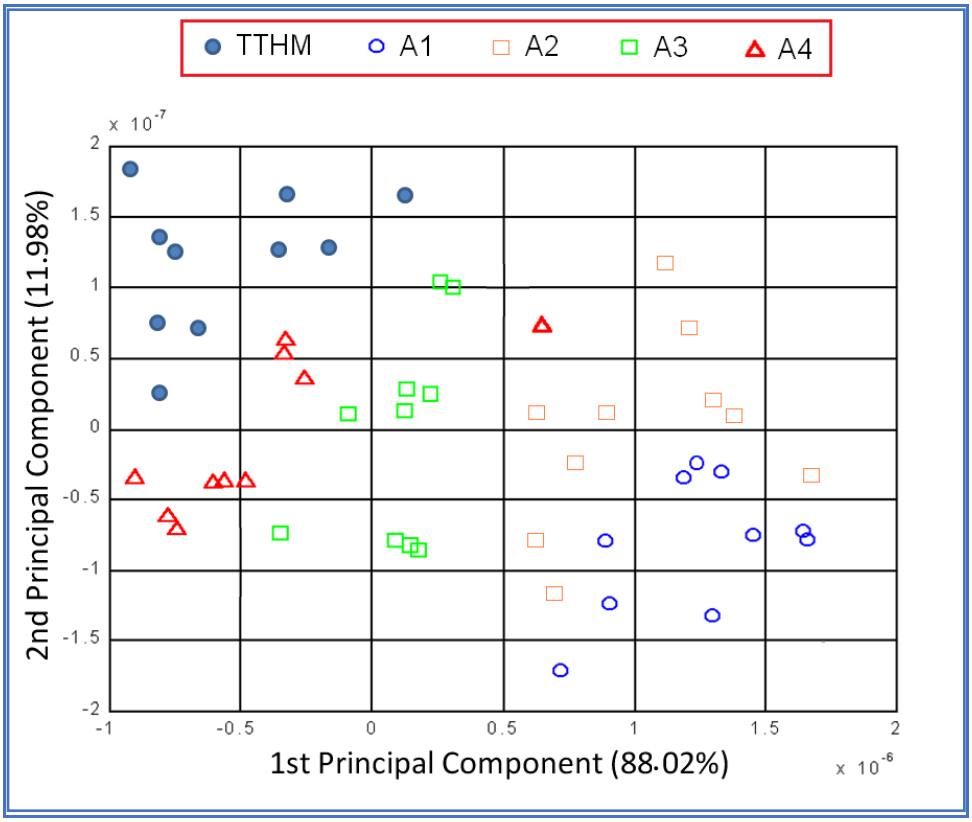


Fig. 8. PCA graph for the TTHM samples and samples A1, A2, A3 and A4, collected at the São João ranch, for the set composed of 10 sensors.

distinctly, which suggests that the sensors can differentiate samples before and after treatment, as well as solutions containing trihalomethanes. There is a tendency for the results for the A4 sample to be closer to the data for the standard sample of all trihalomethanes (TTHM), which would suggest that the sensors would be behaving similarly for these samples. However, it was not possible to confirm the detection limits for these samples, which were extremely lower than the LD detection limit for the sensors. A possible explanation could be the complexity of sewage effluent samples, which could effectively interfere with the selectivity of the sensors [45;57]. These results suggest the need for an improvement in the sensory system, aiming at greater selectivity of it, in an attempt to achieve a specific device for this type of sample.

Sensory response to contamination experiments

Ultrapure distilled water and BioSepT effluent samples were contaminated in the laboratory with a TTHM solution, in order to observe the response of the sensory system. The different depositions and conditions of the polymers on the interdigitated microelectrodes in response to the different samples were analyzed by PCA, Fig. 9, where clear discrimination between the samples is observed. Plotting the score factors on the same PC1-PC2 plane, a clear separation was observed between the samples contaminated by standard TTHM solution (Fig. 9). The sensory system was able to differentiate them, producing different impedance values in each sensor. The sensitivity and selectivity of the sensors about the samples showed excellent distinction in the first main component. The system responded in a particular way for each sample, according to the interaction of the sensory arrangement.

The value for the first component represented about 82.51% of the total variance of the observations, for the samples contaminated with chloroform. Therefore, a reliability factor is around 80% (Fig. 9a). For samples contaminated with bromoform, the reliability factor of observations was around 62% (Fig. 9b), whereas, for samples contaminated with

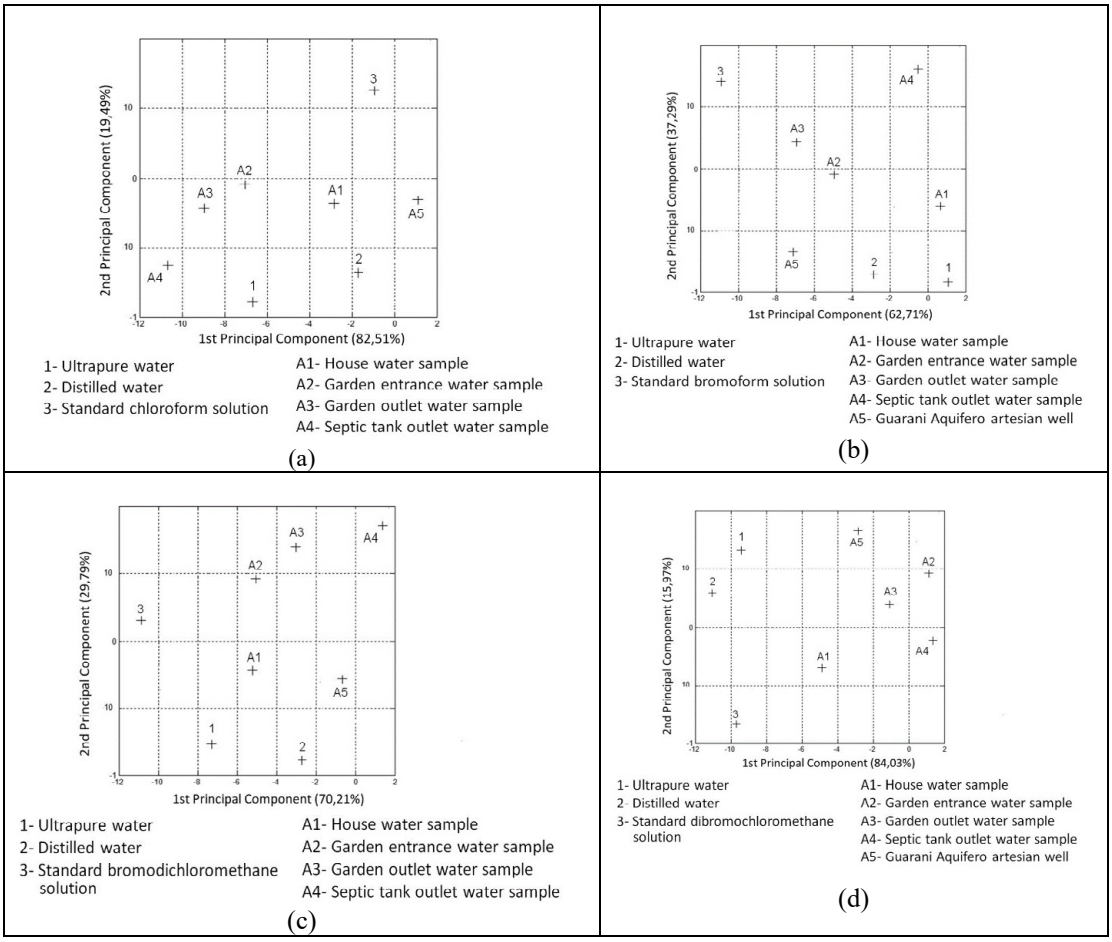


Fig. 9. Principal Component Analysis (PCA) for septic tank effluent samples, ultrapure and distilled water, contaminated with TTHM (s). In (a) samples contaminated with chloroform; in (b) samples contaminated with bromoform; in (c) samples contaminated with bromodichloromethane and, in (d) samples contaminated with dibromochloromethane.

bromodichloromethane and dibromochloromethane, the reliability factors were in the order of 70% and 84% (Fig. 9c and 9d, respectively).

Fig. 10 shows the sensory analysis obtained for samples of ultrapure water, distilled water, and effluents from the BioSepT (sample A3), contaminated with the standard TTHM sample. By plotting the scoring factors on the same PC1-PC2 plane, a clear separation was observed between the samples. The sensory system was, therefore, able to differentiate the water samples from the effluent samples, having a good distinction in the first main component. The sensors responded in a particular way for each sample, according to the interaction of sensory arrangement, sample type, and TTHM solution standard.

The values for the first and second components represent approximately 100% of the total variance of the observations. In Fig. 10, it was obtained for PC1 = 98.02% and for PC2 = 1.98%. These results suggest that sensors respond selectively when in contact with TTHM-contaminated solutions.

Fig. 11, shows that the sensors have excellent selectivity, where the measurement of ultrapure water is not confused with the measurements of the effluent samples from the BioSepT. The PCA analysis showed that the values for the first and second components represent approximately 92% of the total variance, with PC1 = 92.01%; PC2 = 7.99%) of the observations. These results suggest that the sensors respond selectively, when in contact with the solutions, with reliability of almost 90%.

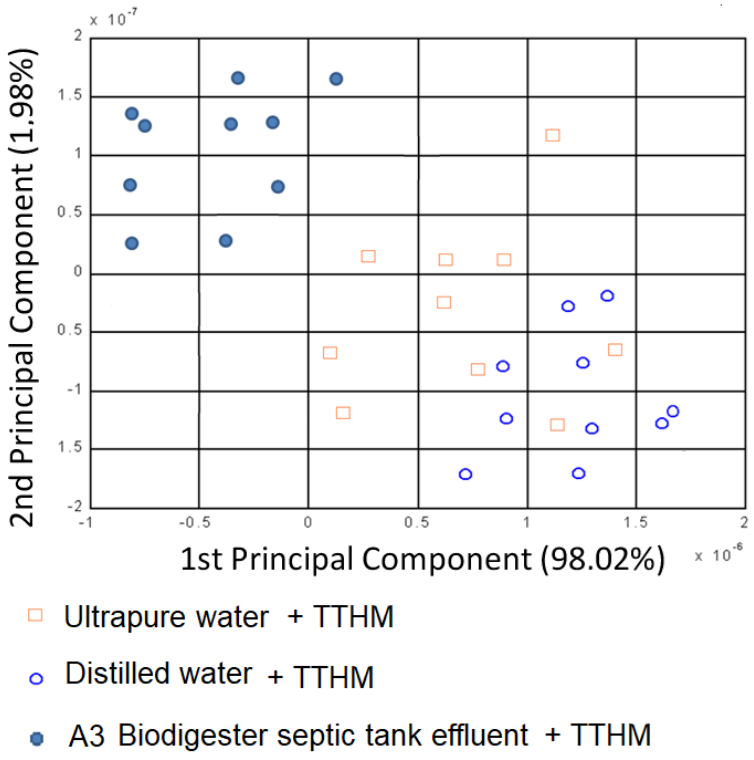


Fig. 10. Sensory analysis obtained for samples of ultrapure water, distilled water and effluents from a biodigestor septic tank (sample A3) contaminated by TTHM.

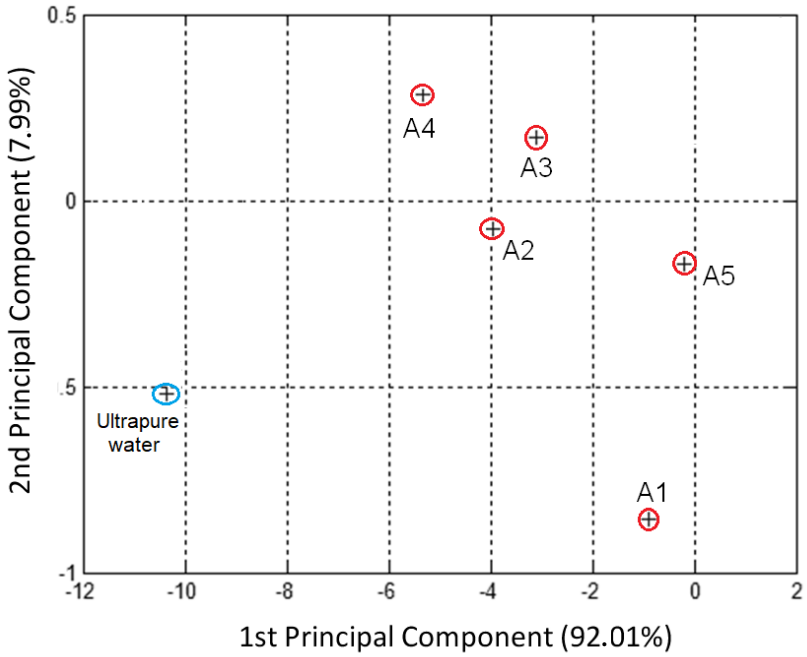


Fig. 11. Analysis of the Main Component (PCA) for effluent samples from a biodigestor septic tank, tap water, artesian well and ultrapure water included in the system.

CONCLUSIONS

This work tested electrochemical sensors made of nanostructured conductive polymers, in treated sewage effluents and contamination tests, based on electrochemical impedance, in order to determine their sensitivity for the detection of carcinogenic compounds, such as THM(s). Describes a new proposal for the evaluation and differentiation of treated sewage effluents, through a sensory system based on global selectivity, allowing the statistical discrimination of the samples. The Polymeric films interacted selectively and allowed different signals for each sample, containing standard TTHM solution. The use of the system, in the evaluation of effluents from a BioSepT, is, therefore, promising. More tests are needed, but future expectations are to update the system and implement monitoring of nanostructured sensors based on reuse domestic effluents, mainly in agriculture or for other purposes, in a sustainable way.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from CNPQ, FINEP, EMBRAPA- REDE AGRONANO and CAPES, for the fellowship.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Ibrd-Ida-The World Bank, 2020. "Como está a América Latina em ternos de Saneamneto? ". Disponível em <https://www.iadb.org/en/improvinglives/how-latin-america-terms-sanitation>. Acesso em: 10/06/2021.
2. World Bank, 2016. América Latina tem água em abundância, mas falta saneamento. Disponível em: <https://saudeamanha.fiocruz.br/america-latina-tem-agua-em-abundancia-mas-falta-saneamento/#.YMIQWvlKi3I>. Acesso em: 10/06/2021.
3. ONU, 2006. Meeting the mdg drinking Water and sanitation The urban and rural challenge of the decade. World Health Organization and UNICEF 2006. WHO Press, ISBN 92 4 156325 7, 44p.
4. Nuvolari, Ariovaldo. Esgoto Sanitário: coleta, transporte, tratamento e reuso agrícola.2. ed. São Paulo: Blucher, 2011. 565 p.
5. Mara, D.; Cairncross, S. Guidelines for the safe use of wastewater and excretain agricuture and aquaculture: Measures for public health protection. Geneva: World Health Organization, 1989, 187 p.
6. Azevedo, L. P., de Oliveira, E. L., 2005. Efeitos da Aplicação de Efluente de Tratamento de Esgoto na Fertilidade do Solo e Produtividade de Pepino Sob Irrigação Subsuperficial. Eng. Agríc., Iaboticabal. v.25, n.1, p.253-263, jan. /abr. 2005, p.253-263.
7. Swartz, L.S., 1999. A compuier water balance model for the evaluation of slow-rate hmd apptication systems in Florida. NET, jan. 1999. Disponível em: http: //www2.dep.state.fl.us/water/f/dom/landap98.htm>. Acesso em: 20 de april de 2021.
8. Zambon, J., 2011. Utilização de águas residuais na irrigação de clones de Eucalipto. /. – Rio Verde – GO.: FESURV, 2011. 24f.: 29,7cm.
9. Biswas, A. K., 1988. Role of wastewater reuse in water planning and management. In: Treatment and Reuse of Wastewater, Ed. Butteworths, 1988.
10. Duarte AS, Airoldi RPS, Folegatti MV, Botrel TA, Soares TM. Efeitos da aplicação de efluente tratado no solo: pH, matéria orgânica, fósforo e potássio. Revista Brasileira de Engenharia Agrícola e Ambiental. 2008;12(3):302-10.
11. Bond WJ. Effluent irrigation—an environmental challenge for soil science. Soil Research. 1998;36(4):543.
12. Environmental Protection Agency, EPA, 68 FR 49547: National Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule; National Primary and Secondary Drinking Water Regulations: Approval of Analytical Methods for Chemical Contaminants. 03-18149 ed. [s/i]: Federal Register, 2003. 135 p., Disponível em: <https://www.govinfo.gov/content/pkg/FR-2003-08-18/pdf/0318149.pdf>. Acesso em: 11 april 2021.
13. Environmental Protection Agency, EPA, 71 FR 387: National Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule. 06-3 ed. [s/i]: Federal Register, 2006. 107 p. Disponível em: <https://www.govinfo.gov/content/pkg/FR-2006-01-04/pdf/06-3.pdf>. Acesso em: 11 april 2021.
14. Environmental Protection Agency, EPA, Rule nº 816F02021, de 1998. Drinking Water Requirements for States and Public Water Systems: Stage 1 and Stage 2 Disinfectants and Disinfection Byproducts Rules. United States, 2001.
15. Environmental Protection Agency, EPA. Office of Environmental Enforcement. Water treatment manual: Desinfection. Wexford: EPA, 2011. 200 p. Disponível em: <https://www.epa.ie/pubs/advice/drinkingwater/Disinfection2_web.pdf>. Acesso em: 11 april 2021.
16. Daniel, L.A., 2001. "Processos de Desinfecção e Desinfetantes Alternativos na Produção de Água Potável". Rio de Janeiro: RiMa, ABES, 2001, 155p (PROSAB).
17. Tchobanoglons, G.; Bmton, F L; Stensel, R.D., 2003. Wastewater engineering, treatment and reuse. 4 th ed./ revised. Nova Iorque: Metcalf & Eddy me. McGraw-Hill, p. 1819.
18. Bolzonella D, Fatone F, Pavan P, Cecchi F. Anaerobic Fermentation of Organic Municipal Solid Wastes for the Production of Soluble Organic Compounds. Industrial & Engineering Chemistry Research. 2005;44(10):3412-8.
19. Bouwer, R, Fox: P., Westerhoff, P., 1998. Irrigation with Treated Effluent How this prnctice aflect underlying groundwater. Water Enviromnent and Technology.
20. Von Sperling, M., 1995. Introdução à qualidade das águas e ao tratamento de esgoto. Belo Horizonte: Departamento de Engenharia Sanitária e Anibiental- Universidade FederaI de Minas Gerais, 243p.
21. DeAngelo AB, Geter DR, Rosenberg DW, Crary CK, George MH. The induction of aberrant crypt foci (ACF) in the

colons of rats by trihalomethanes administered in the drinking water. Cancer Letters. 2002;187(1-2):25-31.
22. Brasil Leis, decretos, etc ... Portaria n 36, de 19 de janeiro de 1990.
23. Komulainen H. Experimental cancer studies of chlorinated by-products. Toxicology. 2004;198(1-3):239-48.
24. Fawell, l., 2000. Food Chemistry Toxicology. Food and Chemical Toxicology, v.38, n.l, p.91-95.
25. Goncalves, R. F., 2003. Desinfecção de Efinentes Sanitários., 1 ed. São Carlos/SP: RiMa Artes e Textos, v.l, 303p (PROSAB).
26. Tsitsifli S, Kanakoudis V. Disinfection Impacts to Drinking Water Safety—A Review. Proceedings. 2018;2(11):603.
27. Rebhun M, Heller-Grossman L, Manka J. Formation of disinfection byproducts during chlorination of secondary effluent and renovated water. Water Environment Research. 1997;69(6):1154-62.
28. Zilda Borsoi; Laruni, G. M. L. C. N.; Gomes, S. T. S. M., 1997. Tratamento de esgoto: tecnologias acessíveis informe infra-estrutura área de projetos de infra-estrutura, novembro/97 n.16, 8p.
29. Schmitt, F.; Weschenfelder, S.; Vim, T. M., 2006. Florianópolis, junho/06. Tratamento anaeróbio de efluentes. G7416.DOC 29/0l/2002, 8p.
30. Acher A, Fischer E, Turnheim R, Manor Y. Ecologically friendly wastewater disinfection techniques. Water Research. 1997;31(6):1398-404.
31. Novaes, AP.; Simões, M.L.; Martin-Neto, L.; Crnvínel, P.E.; Santana, A; Novotny, E. R; Santiago, G.; Nogueira, ARA, 2002. "Utilização de uma fossa séptica para melhoria do saneamento rural e desenvolvimento da agricultura orgânica". Comunicado Técnico 46. Embrapa Instrumentação Agropecuária, São Carlos, 2002.
32. Schoken-lturrino, RP.; Benincasa, M; Lucas Jr., J.; Felis, S. D., 1995. "Biodigestores continuos: isolamento de bactérias patogênicas no efluente". Engenharia Agrícola, v. 15, p. 105-108, 1995.
33. WEF - Water Envimnmenta1 Federntion. Wastewo.ter disinfection: manual of pratice FD-10. Alexandria: WEF, 1996.
34. Rossi Léo, L. F.; Pivelli, R P.; Fabretti, A. A; Macedo, M. F., 2005. Avaliação da formação de trihalometanos na desinfecção de esgotos brutos, anaeróbios, facültativos e pás-trntados com hipoclorito de sódio. 3° Congresso Brnsileiro de Engenbarla Sanitária e Ambiental - Campo Grnnde ABES - Associação Brasileirn de Engenharia Sanitária e Ambiental, 11p.
35. De Luca, S. 1.; Reggio, E., 2003. Desinfecção de efiuentes tIatados com hipoclorito de sódio. In: CONGRESSO BRASILEIRO DE ENGENHARIA SANITÁRIA E AMBIENTAL, 22., Joinville, 2003.Anais ... Rio de Janeiro: l\RES, 2003.
36. OMS, 1989. Diretrices sanitarias sobre el uso de aguas resíduales en agricultura y acuicultura. Ginebra: Organización Mundial de Ia Salud, 1989. 65 p. (Serie Informes Tecnicos 778).
37. Barisci, 1. N.; Conn, C.; Wallage, G. G., 1996. Conducting polymer sensors. Trends in Polymer Science, 4 (9), p. 307.
38. Barraud, A., 1990. Chemical sensors based on LB films. *Vaccum*, 41 (7-9), p.1624 (1990).
39. Riul Jr.A., H.C. Souza, R.R. Malmegrim, D.S. Santos Jr., A.C.P.L.F. Carvalho, F.J. Fonseca, O.N. Oliveira Jr., L.H.C. Mattoso, 2004. Nanostructured films of perylene derivatives: High performance materials for taste sensor applicationsSens. Act. B, 98: 77-82.
40. Krantz-Rülcker C., M. Stenberg, F. Winquist, I. Lundström, 2001. Electronic tongues based on sensor arrays and pattern recognition: a reviewAnal. Chim. Acta, 426: 217-226.
41. Charef A, Ghauch A, Baussand P, Martin-Bouyer M. Water quality monitoring using a smart sensing system. Measurement. 2000;28(3):219-24.
42. Mortimer RJ, Dyer AL, Reynolds JR. Electrochromic organic and polymeric materials for display applications. Displays. 2006;27(1):2-18.
43. Consolin Filho, N.; Venancio, E. C; Carvalho, E. R.; Santino, M. B. C.; Bianchini JR., I.; Mattoso, L. H. C., 2008. Sensors & Transducers. Eutrophic Level Evaluation of Aquatic Environments Using a Sensorial System Based on Nanostructured Conducting Polymers, 93:92-102.
44. Carvalho, E. R.; Consolin Filho, N.; Firmino A; Oliveira JR., O.N; Mattoso, L. H. C; Martin Neto, L., 2006. Sensorial System for Analysis of Chloroform in Water. Sensor Letters,4: 129-134.
45. Carvalho, E. R. and Silva, W. T. L., (2016). Evaluation of Tap Water Based on Sensor Array and Conducting Nanostructured Polymers. J. Water Environ. Nanotechnol., 1(2): 116-123, Autumn.
46. Janata, J., 1989. Principles of Chemical Sensors; Plenum Press: New York, 1989.
47. Zhang Q, Jin H, Wang X, Jing X. Morphology of conductive blend fibers of polyaniline and polyamide-11. Synthetic Metals. 2001;123(3):481-5.
48. Mattoso LHC, Manohar SK, Macdiarmid AG, Epstein AJ. Studies on the chemical syntheses and on the characteristics of polyaniline derivatives. Journal of Polymer Science Part A: Polymer Chemistry. 1995;33(8):1227-34.
49. Macdiarmid, A.G.; Yang, L.S.; Hang, W.S.; Humphrey, B.D., 1987. Synth. Met., v.18, p. 393.
50. Carvalho ER, Martin-Neto L, Milori DMBP, Rocha JC, Rosa AH. Interactions of chlorine with tropical aquatic fulvic acids and formation of intermediates observed by fluorescence spectroscopy. Journal of the Brazilian Chemical Society. 2004;15(3):421-6.
51. Aiken, G. R., 1985. Humic Substances in Soil, Sediment and Water; John Wiley & Sons: New York.
52. Malcolm, R. L., 1989. Humic Substances in the Aquatic and Terrestrial Environment; Springer-Verlag: Berlim.
53. Paterno LG, Constantino CJL, Oliveira ON, Mattoso LHC. Self-assembled films of poly(o-ethoxyaniline) complexed with sulfonated lignin. Colloids and Surfaces B: Biointerfaces. 2002;23(4):257-62.
54. Crespilho FN, Zucolotto V, Siqueira JR, Constantino CJL, Nart FC, Oliveira ON. Immobilization of Humic Acid in Nanostructured Layer-by-Layer Films for Sensing Applications. Environmental Science & Technology. 2005;39(14):5385-9.
55. Raposo M, Oliveira Jr ON. Adsorption mechanisms in layer-by-layer films. Brazilian Journal of Physics. 1998;28(4):00-.
56. Carvalho E, Consolin Filho N, Venancio E, O O, Mattoso L, Martin-Neto L. Detection of Brominated By-Products Using a Sensor Array Based on Nanostructured Thin Films of Conducting Polymers. Sensors. 2007;7(12):3258-71.
57. Riul A, dos Santos DS, Wohnrath K, Di Tommazo R, Carvalho ACPLF, Fonseca FJ, et al. Artificial Taste Sensor: Efficient Combination of Sensors Made from Langmuir–

Blodgett Films of Conducting Polymers and a Ruthenium Complex and Self-Assembled Films of an Azobenzene-Containing Polymer. Langmuir. 2002;18(1):239-45.
58. Ivarsson P, Holmin S, Höjer N-E, Krantz-Rülcker C, Winquist F. Discrimination of tea by means of a voltammetric electronic tongue and different applied waveforms. Sensors and Actuators B: Chemical. 2001;76(1-3):449-54.
59. Toko K. Taste sensor. Sensors and Actuators B: Chemical. 2000;64(1-3):205-15.
60. Almeida-Neto, A. S.; Freitas, D. de; Lugli, L. V. B.; M. H.; Fernandes, M. A.; Almeida, R. C.; Santos, S. A. M., 202). Atlas histórico e socioambiental das regiões hidrográficas de São Carlos-SP. São Carlos-SP: Diagrama Editorial 2020, 166p., ISBN: 978-65-86512-00-7.
61. Silva FLd, Picharillo C, Macera LG, Monteiro JDA, Peres RB. Procedimentos para a obtenção do pagamento por serviços ambientais no âmbito da instituição de uma reserva particular do patrimônio natural. Extensão Rural. 2016;23(3):120.
64. Iniciativa Verd, 2015. Relatório: um milhão de árvores nativas em dez anos de vida Fevereiro de 2015, 36p.
65. Hernandes, P., 2021. Empresa Brasileira de Pesquisa Agropecuária. Soluções tecnológicas, Fossa séptica biodigestora. Disponível em:<https://www.embrapa.br/busca-de-solucoes-tecnologicas/-/produto-servico/721/fossa-septica-biodigestora. Acesso em: 02/04/2021.